Protein Science (1995), 4:1017–1019. Cambridge University Press. Printed in the USA.

RECOLLECTIONS

One-gene–one-enzyme: Remembering biochemical genetics

NORMAN H. HOROWITZ

Biology Division, California Institute of Technology, Pasadena, California 91125

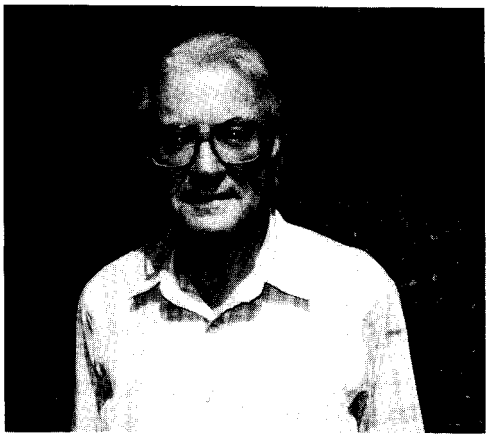
The discovery that proteins are encoded in the genes, which are themselves not proteins, has long seemed to me to be the key to understanding the organization of living matter. To be able to state with confidence that the genetic part of the organism—the part that is transmitted from generation to generation—consists of instructions in the form of DNA for the synthesis of proteins, which later produce and operate the organism, implies a depth of knowledge that would have astonished biologists of an earlier day. In their *Enzymes* (3rd edition, 1979), Dixon and Webb called this insight "probably the most important discovery ever made in biology." I agree.

This discovery rests on the work of many scientists of the 19th and first half of the 20th centuries, most of whom did not live to see the outcome of their efforts. I am a survivor who was lucky enough to work in one of the laboratories—that of Beadle and Tatum at Stanford University in the 1940s—that contributed to the grand conclusion and who has lived to see its general acceptance. This essay recalls the early years of what we called "biochemical genetics"—roughly, the decade preceding the discovery of the structure of DNA. Following the DNA breakthrough, biochemical genetics became part of the science of molecular genetics, which then came into existence.

I learned genetics in the first half of the century. Thomas Hunt Morgan was chairman of my Ph.D. oral committee, in 1939. I can remember the genetics of those days—a specialized science with its own language and its own units, only weakly connected to the rest of biology, not widely considered to be very important, let alone central, to biology as a whole. It was often treated as a form of applied biology and was associated with plant and animal breeding departments.

That picture began to change in 1935. In that year, George Beadle and Boris Ephrussi, having met at Caltech, moved to Ephrussi's laboratory in Paris, where they planned to grow imaginal discs from *Drosophila* in tissue culture in order to study the role of genes in development. Their attempts to culture the discs in vitro failed, but they succeeded in transplanting them from one embryo to another, where they continued to develop. This operation, applied to various eye-color mutants, enabled them to demonstrate that formation of the brown component of *Drosophila* eye color involves two substances, one of them the precursor of the other. The production of each of the substances is controlled by a different gene. Eventually, kynurenine and OH-kynurenine were identified as the two substances, with kynurenine preceding OH-kynurenine in the chain. The latter is a precursor of the brown pigment. This was the first demonstration that, in biosynthetic reaction chains, different steps are controlled by different genes. It suggested great questions that needed to be explored.

The development of *Drosophila* was biochemically too mysterious to provide a route for further explorations of gene action. A different kind of system was required. It was at this point that Beadle, who was then on the faculty of Stanford University, attended a lecture on the nutrition of microbes by Edward



Norman H. Horowitz.

Reprint requests to: Norman H. Horowitz, Biology Division 156-29, California Institute of Technology, Pasadena, California 91125.

Norman H. Horowitz was born in Pittsburgh, Pennsylvania in 1915. He studied biology at the University of Pittsburgh (B.S., 1936) and the California Institute of Technology (Ph.D., 1939). He went to Stanford University as a National Research Council Fellow in 1939. There he met George Beadle, and in 1942 he joined Beadle's group to work on the newly discovered biochemical mutants of *Neurospora*. In 1946, Dr. Horowitz returned to Caltech with Beadle, where he joined the faculty and where he has remained until the present time. In the 1960s, he became interested in the exploration of Mars, then being conducted at the Jet Propulsion Laboratory in Pasadena, and from 1965 to 1970 he was chief of its Bioscience Section. Dr. Horowitz was a member of the scientific teams of several missions to Mars, including the Viking lander (1976–1977), and came to the conclusion that there is no present life on the surface of Mars. He is a member of the National Academy of Sciences.

Tatum, then a research associate in Beadle's laboratory. He learned from Tatum that although bacteria are alike in their basic biochemistry, they differ in their growth-factor requirements. If these differences are genetic in origin, Beadle reflected, it should be possible to induce mutations producing them. This inspiration occurred years before it was established that bacteria do, in fact, have genes. Because there was then no genetics of bacteria, Beadle chose another microorganism, the mold *Neurospora*, to test his idea.

The beautifully simple genetic machinery of *Neurospora* was well understood, having been worked out largely by B.O. Dodge at the New York Botanical Garden. Dodge was a close friend of T.H. Morgan and convinced him to take some cultures with him in 1928 when he left Columbia University to found the Biology Division at Caltech. Dodge assured Morgan that *Neurospora* would be an important organism for genetics some day. Morgan gave the cultures to a graduate student, Carl Lindegren, to work on for his thesis. Lindegren made some further contribution to the knowledge of the organism. Beadle had heard Dodge lecture on *Neurospora*, and he had seen it in culture during his postdoctoral years at Caltech. When he decided to look for nutritional mutants in a microorganism, *Neurospora* was naturally the organism he chose.

The experiment consisted of X-raying one parent of a cross and culturing the progeny (haploid ascospores, isolated singly and numbered) on a "complete" medium—i.e., one designed to satisfy the maximal number of nutritional needs, known and unknown. This medium was made by adding yeast and malt extracts to a simple "minimal" medium consisting of salts, sugar, and biotin, the only growth-factor required by wild-type *Neurospora*. Once progeny cultures were obtained, they were tested for their ability to grow on minimal medium. Those that did not grow were further tested to identify their growth requirement.

Beadle told me years later that the chance for success in this venture seemed so low at the time that he and Tatum agreed at the outset to test 5,000 spores before giving up. Success actually came with spore number 299, which grew on minimal medium supplemented with pyridoxine. Crossing to wild type showed that the requirement was inherited, as if caused by mutation of a single gene. Other mutations soon followed number 299, resulting in requirements for various vitamins, amino acids, and nucleic acid bases.

This was the beginning of modern biochemical genetics. Earlier discoveries, most notably those by Cuénot and Garrod, made soon after the rediscovery of Mendelism, had shown a connection between Mendelian factors and biochemical reactions. These findings had been all but forgotten as time passed, however. The chief reason for this neglect is, I believe, the fact that there was no suitable experimental organism in which the early observations could be pursued. Geneticists studied those aspects of their subject that were accessible with the means available, and these aspects did not include the biochemical one. Beadle's inspiration to search for nutritional mutants in a microorganism that grew on a synthetic medium was simply a stroke of genius. It opened a new world.

I was deeply impressed when I heard Beadle describe their early findings at a seminar at Caltech in 1941, and I gladly accepted an invitation to move to his laboratory at Stanford to participate in the work. I remained there until 1946, when the lab broke up. During those exciting years, the major tasks were to identify the reactions that were abolished in the mutants, causing them to exhibit new nutritional requirements, and to confirm that they resulted from single-gene changes. Although most of our interest was in this work, these were war years, and significant effort was devoted to applications, such as the development of bioassay methods, for which the mutants were useful. The most important scientific output of those years was, notwithstanding, the assignment of individual genes to specific steps in biosynthetic pathways. In some cases, the pathway was not known before gene assignments were made, and it had to be discovered; in others, the pathway was known or suspected, and the genes controlling the steps had to be assigned.

The resulting evidence allowed Beadle in 1945 to propose the famous one-gene–one-enzyme hypothesis (later expanded to one-gene–one-protein), which held that each gene involved in biosynthetic pathways controls the synthesis of a single enzyme. This idea was already hinted at in the first *Neurospora* paper of Beadle and Tatum (1941) as a possibility, but it took several years of laboratory investigation before sufficient data were accumulated to support it publicly. A few mutants appeared to contradict the idea. These were of special interest and became the subjects of lengthy investigations.

The first example was a mutant that initially appeared to require a new amino acid in casein, because it grew when supplied casein hydrolysate but not when given a mixture of all the known amino acids of protein. Tatum and David Bonner set out to isolate and identify the new substance, which came to be known in the lab as "neurosporine." They found that neurosporine was not a new amino acid, but a mixture of isoleucine and valine in the particular ratio that occurs in casein. Growth was inhibited at other ratios. There were no new amino acids to be discovered in casein by 1942, but there was the puzzle of a single-gene mutant with a double amino acid requirement. After much work and several false leads, it was eventually shown by J. Myers and E. Adelberg that this gene does not encode two enzymes, a different one for each amino acid, but only one. The same set of enzymes, they found, are used to catalyze the final steps in the syntheses of isoleucine and valine.

Another example was a mutant that required both methionine and threonine for growth. In 1946, a graduate student, Howard Teas, tried but failed to separate the two requirements genetically—his findings showed that they probably resulted from the mutation of one gene. Among the compounds Teas tested on the mutant, he had the inspiration to try homoserine. The mutant grew. It was blocked in the synthesis of this compound which, we learned, is at a branch point in metabolism: one branch leads to threonine and the other to cystathionine. The latter, I had found earlier, is a precursor of methionine.

The demonstration that these cases did not contradict the one-gene–one-enzyme principle, but in fact supported it, increased one's confidence in the idea. In spite of the evidence in its favor, however, it proved to be indigestible to probably most geneticists, and they refused to accept it. I still have a photograph of a two-headed calf that was sent to me in 1951 by Joshua Lederberg. One head is labeled Methionine and the other Cysteine. The caption reads "Replica of a multifunctional monogenic enzyme. Compliments of the Department of Genetics, University of Wisconsin." The idea of a fundamental simplicity in gene action was too radical for the times. I think now that nothing short of a complete account of the role of genes in protein synthesis would have satisfied our critics, but such an accounting was still some years away.

With one exception, the objections to the hypothesis consisted of vaguely expressed feelings of doubt and skepticism. The exception was a criticism advanced by Max Delbrück at Cold Spring Harbor in 1946. Delbrück argued that the mutants we had studied and that formed the basis for the hypothesis were not representative of mutants as a whole. The requirement that all mutants must grow on complete medium was selective because it excluded any mutants that require nondiffusible molecules. Mutants of genes that control many enzymes would have many growth requirements, at least one of which would likely be nondiffusible. Such mutants would not grow on complete medium. Our method for detecting mutations thus excluded a whole class of mutants, and the one-gene–one-enzyme hypothesis was consequently based on mutants selected to support it.

This argument was not very strong because even our biased selection procedure should have picked up multifunctional genes whose mutants happened to be satisfied by complete medium. Yet, as I explained above, study of the two such genes that had been found up to then showed that both were the kind of exception that prove the rule. Delbrück's argument posed an interesting problem, however—how to minimize the selective action of the growth medium—and in any case it seemed important to answer it for the sake of the theory.

It occurred to me that the question raised by Delbrück could be answered by the use of temperature-sensitive mutants. These mutants show their mutant character only at certain temperatures; at other temperatures, they are like wild type. They were first observed following a series of events involving historic mutant No. 299.

Beadle received a letter from an acquaintance of his at the Merck Research Laboratory shortly after publication of the 1941 paper describing the mutant. The letter requested a transfer of No. 299 for the purpose of developing an assay method for pyridoxine. Beadle sent the mutant (he never withheld a mutant once it had been mentioned in print). A few months later, Beadle read a letter to us at the afternoon tea-break that had come from his friend at Merck. It said that they (Stokes, Foster, and Woodward) had found that No. 299 would grow on minimal medium if the pH of the medium was raised to 6 from its normal value, 5.

Such a role for an environmental variable in the expression of a mutant trait was a revelation to us, and we agreed that afternoon to add another step to the mutant hunt that ran continually in the lab. This step consisted of an incubation at 35 °C on minimal medium in addition to the usual one at 25 °C; its purpose was to learn if temperature might also be important for the expression of mutations. The result was the discovery of temperature-sensitive mutants. The majority of these, but not all, were phenotypically mutant at 35 °C and wild at 25 °C. Their nutritional requirements, where known, covered all the major classes of ordinary mutants—i.e., amino acids, vitamins, and nucleic acid bases—and some of them were found to be alleles of the usual non-temperature-sensitive mutants. Later, they were found to produce thermally unstable enzymes.

These mutants offered a means of answering the question raised by Delbrück. They could be recovered at 25 °C, where they grew like wild type, and tested for their growth requirement at 35 °C, where they behaved as mutants. Mutants that required nondiffusible substances, or substances not present in complete medium—those that would be selected against in the standard mutant-hunt procedure—would fail to grow on complete medium at 35 °C. If the fraction of these was large, then Delbrück's criticism was justified. If it was small, however, then the criticism lost its force. At the time, we had 26 temperature-sensitive mutants, and of these, 14 grew on complete medium and 12 did not at 35 °C. From these rather small numbers it appeared that the class of mutants on which the one-gene–one-enzyme theory was based was not a small fraction, but made up something like half of all mutants.

In order to increase the number of temperature-sensitive mutants, Urs Leupold and I, now back at Caltech, undertook a mutant hunt in *Escherichia coli*, which Tatum had shown produces biochemical mutants similar to those of *Neurospora*. Leupold devised an ingenious method for detecting the mutants on a large scale, and before long he had 161 of them. The fraction of these that grew on complete medium at 40 °C was 0.77. The one-gene–one-enzyme hypothesis was safe. We presented these results at Cold Spring Harbor in 1951, but by then Delbrück had lost interest in the problem and was not in the audience. I think that from the beginning he was less interested in the answer to the problem he had posed than in how an answer might be reached. Once the principle was demonstrated in *Neurospora*, he didn't care about the bacterial expansion.

The older I get, the more I appreciate the difference between the results of scientific investigations and the methods by which the results are obtained. The results constitute the body of scientific knowledge—they *are* science. But, as has been noted by others, the facts of science exist in nature and are waiting to be discovered; if not found by one investigator, they will be found by another. The methods, however, are the creations of individual scientists; they are more art than science. For this reason, and depending on one's mood, they may be even more admirable than the science they make possible. The history of biochemical genetics provides many examples, large and small, of art and science.